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## **A short bout of HFD promotes long-lasting hepatic lipid accumulation**

Chiazza, Fausto ; Challa, Tenagne D ; Lucchini, Fabrizio C ; Konrad, Daniel ; Wueest, Stephan

**Abstract:** A short bout of high fat diet (HFD) impairs glucose tolerance and induces hepatic steatosis in mice. Here, we aimed to elaborate on long-lasting effects of short-term high fat feeding. As expected, one week of HFD significantly impaired glucose tolerance. Intriguingly, recovery feeding with a standard rodent diet for 8 weeks did not fully normalize glucose tolerance. In addition, mice exposed to a short bout of HFD revealed significantly increased liver fat accumulation paralleled by elevated portal free fatty acid levels after 8 weeks of recovery feeding compared to exclusively chow-fed littermates. In conclusion, a short bout of HFD has long-lasting effects on hepatic lipid accumulation and glucose tolerance.

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BRIEF REPORT

## A short bout of HFD promotes long-lasting hepatic lipid accumulation

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### ABSTRACT

A short bout of high fat diet (HFD) impairs glucose tolerance and induces hepatic steatosis in mice. Here, we aimed to elaborate on long-lasting effects of short-term high fat feeding. As expected, one week of HFD significantly impaired glucose tolerance. Intriguingly, recovery feeding with a standard rodent diet for 8 weeks did not fully normalize glucose tolerance. In addition, mice exposed to a short bout of HFD revealed significantly increased liver fat accumulation paralleled by elevated portal free fatty acid levels after 8 weeks of recovery feeding compared to exclusively chow-fed littermates. In conclusion, a short bout of HFD has long-lasting effects on hepatic lipid accumulation and glucose tolerance.

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### Introduction

A short bout of high fat diet (HFD) induces adipose tissue inflammation, glucose intolerance and hepatic steatosis.<sup>1–5</sup> While glucose intolerance mainly results from impaired hepatic insulin sensitivity, increased hepatic fat accumulation upon short-term HFD may evolve due to elevated circulating free fatty acid (FFA) levels provoked by dietary fat supply.<sup>1,3,5,6</sup> Likewise, plasma FFA were shown to be the main source of fatty acids bound in liver triglycerides in manifested obesity.<sup>7</sup> However, in contrast to short-term HFD-induced liver steatosis, adipose tissue lipolysis is thought to be the main source of circulating FFA leading to liver fat accumulation upon chronic caloric surplus.<sup>8</sup> Accordingly, lipolytic activity in mesenteric adipose tissue may be of high relevance in the latter, as mesenteric fat is mainly drained via the portal vein to the liver.<sup>9</sup> In support of such notion, increased visceral lipolysis was linked to obesity-induced fat accumulation in morbidly obese humans.<sup>10</sup>

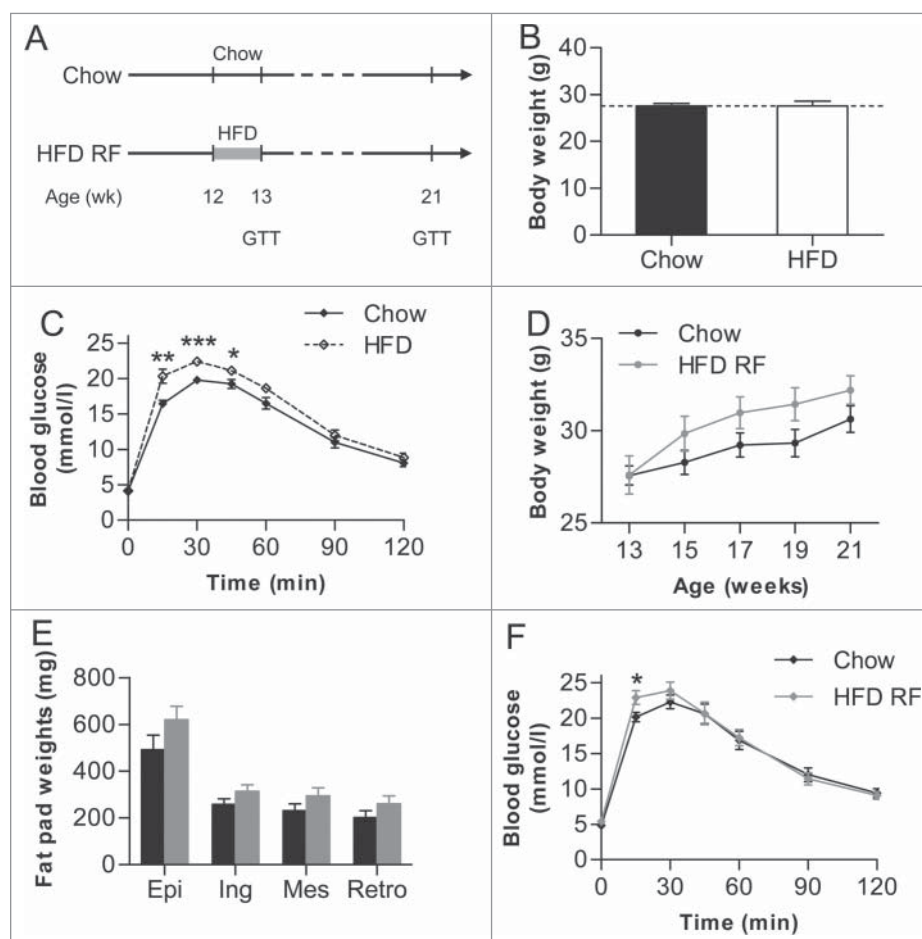
The negative impact of short-term high fat feeding on glucose metabolism and liver fat accumulation is well established. However, it remains unclear whether such effects are readily reversible or rather persistent. In order to elaborate on potential long-standing effects of short-term HFD on metabolism, C57BL/6J mice received a short bout of HFD before being placed on a standard rodent diet (chow) for 8 weeks (recovery feeding (RF)) (Fig. 1A). We provide evidence that a short HFD challenge is sufficient to induce imprinting effects on glucose

metabolism. In addition, short-term high fat feeding has long-lasting effects on portal free fatty acid levels as well as liver lipid accumulation.

### Results

C57BL/6J mice were fed either a high fat or chow diet for one week. As depicted in Figure 1B, short-term high fat diet (HFD) had no effect on body weight. In contrast and as expected, glucose tolerance was significantly deteriorated in HFD compared to chow-fed littermates (Fig. 1C) (AUC in chow-fed mice  $1682 \pm 57 \text{ mmol/l}^* \text{ min}$  vs. AUC in HFD-fed mice  $1895 \pm 43 \text{ mmol/l}^* \text{ min}$ ;  $p < 0.05$ ). Subsequently, all mice were fed a chow diet for 8 weeks to investigate long-lasting effects of a short bout of HFD. As shown in Figure 1D, body weight gain during the follow-up was slightly higher in the HFD-fed group, as was total body weight at the end of these 8 weeks ( $30.6 \pm 0.7$  vs.  $32.2 \pm 0.8$ ,  $p = 0.17$ ). Similarly, fat pad weights did not significantly differ, although all depots analyzed tended to be heavier in the group that was previously exposed to a fat enriched diet (Fig. 1E). Intriguingly, 8 weeks of RF was not enough to fully normalize glucose intolerance induced by short-term HFD (Fig. 1F). Hence, a short bout of HFD may have long-lasting effects on glucose homeostasis.

We and others have previously shown that a short bout of HFD induces TNF $\alpha$  expression in white adipose tissue.<sup>1,2,4,5</sup> It was previously suggested that such rise in

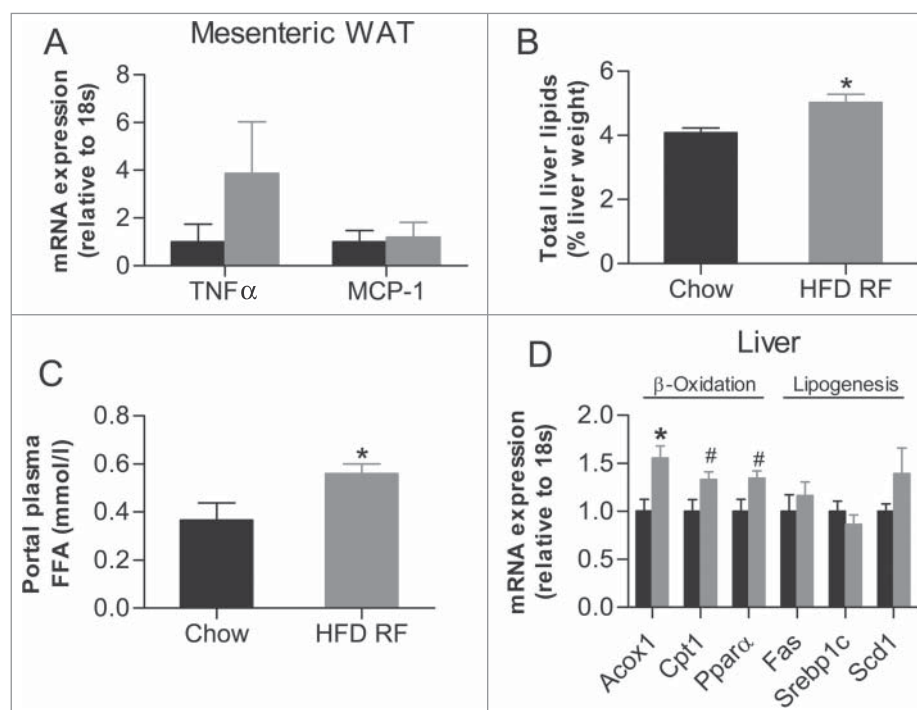


**Figure 1.** Long-lasting effect of short-term HFD on glucose tolerance. (A) Study design. (B) Body weights of chow-fed (black bars) and HFD-fed (white bars) mice after 1 week of dietary intervention.  $n = 6-8$ . (C) Intraperitoneal glucose tolerance test was performed after 1 week of dietary intervention in chow-fed (●) and HFD-fed (○) mice.  $n = 6-7$ . Body weights (D) and fat depot weights (E) of chow-fed (black bars) and HFD recovery fed (HFD RF; gray bars) mice after 8 weeks of recovery feeding.  $n = 6-8$ . (F) Intraperitoneal glucose tolerance test was performed in chow-fed (●) and HFD recovery fed (HFD RF) (○) mice after 8 weeks of recovery feeding.  $n = 6-8$ . All error bars represent SEM. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$  (Student's  $t$  test).

TNF $\alpha$  may be responsible for observed hepatic derangements induced by short-term HFD.<sup>1</sup> As depicted in **Figure 2A**, TNF $\alpha$  expression in mesenteric WAT was ~4-fold higher in HFD RF mice compared to solely chow-fed littermates. In contrast, expression of monocyte chemoattractant protein-1 (MCP-1), which was not affected in WAT of short-term HFD mice<sup>5</sup> was similarly expressed in both groups (**Fig. 2A**). Of note, harvested mesenteric WAT may be contaminated by pancreatic tissue.<sup>11</sup> Consequently, expression of the pancreatic marker Ela3b was determined and samples with high expression of Ela3b were excluded from mRNA expression analysis.

To unravel whether a short bout of HFD has long-lasting effects on hepatic steatosis, liver lipid accumulation was assessed. As shown in **Figure 2B**, total liver lipid levels were significantly higher in HFD RF compared to chow-fed littermates. Similarly, liver

triglyceride (TG) levels were slightly higher in HFD RF compared to chow-fed mice (HFD RF  $23.0 \pm 2.0$   $\mu\text{mol/g}$ , chow-fed mice  $19.2 \pm 1.6$   $\mu\text{mol/g}$ ,  $p = 0.16$ ). As up to 60% of FFA bound in liver TGs may arise from plasma FFA,<sup>7</sup> FFA levels in systemic as well as portal plasma were determined next. Whereas FFA levels in systemic circulation were similar between the 2 groups (chow-fed mice  $0.52 \pm 0.03$  mmol/l, HFD RF  $0.43 \pm 0.08$  mmol/l,  $p = 0.3$ ), they were significantly increased in the portal vein of HFD RF compared to solely chow-fed littermate mice (**Fig. 2C**). Besides FFA uptake, liver lipid accumulation is influenced by de novo lipogenesis and  $\beta$ -oxidation.<sup>12</sup> Therefore, hepatic mRNA expression of enzymes involved in these pathways was determined. While expression of lipogenic enzymes was similar between both groups, mRNA expression of the  $\beta$ -oxidation genes *Acox1*, *Cpt1* and



**Figure 2.** Long-lasting effect of short-term HFD on hepatic lipid accumulation. (A) mRNA expression of TNF $\alpha$  and MCP-1 in mesenteric white adipose tissue (n = 4–5); (B) total liver lipids (n = 5–6); and (C) portal FFA levels (n = 6) were determined in chow-fed and HFD recovery fed (HFD RF) mice after 8 weeks of recovery feeding. (D) Hepatic mRNA expression of respective genes was analyzed (n = 5–6). All error bars represent SEM. \*p < 0.05, #p = 0.05 (Student's *t* test). Chow-fed mice: black bars; HFD RF mice: gray bars.

*Ppara* was increased in livers of HFD RF mice (Fig. 2D) suggesting increased respiratory capacity in livers of HFD RF mice.

## Discussion

It is well established that a short bout of high fat feeding is sufficient to induce glucose intolerance as well as hepatic steatosis.<sup>2–4</sup> Here, we provide evidence that such alterations may persist after switching to a standard rodent diet. Accordingly, a recovery feeding period with a chow diet 8 times longer than the HFD exposure did not fully normalize glucose tolerance. Although (hepatic) insulin sensitivity was not assessed in this study, it is likely that impaired liver insulin sensitivity in HFD RF mice was responsible for the observed difference in glucose tolerance, as a short-term HFD mainly affects hepatic insulin sensitivity.<sup>1,3,5</sup> Moreover, the fact of increased liver lipid levels in HFD RF mice may further support the assumption of deteriorated hepatic insulin sensitivity, as liver steatosis is often associated with hepatic insulin resistance.<sup>13</sup>

The finding of increased FFA levels in the portal vein of HFD RF mice suggest that increased delivery of FFA to the liver contribute to elevated liver lipid levels. Such notion is further supported by increased hepatic expression of enzymes involved in  $\beta$ -oxidation pointing toward

increased hepatic respiratory capacity of HFD RF mice.<sup>14</sup> This may reflect an adaptation to increased lipid availability to protect from further accumulation of hepatic lipids.<sup>15</sup> But why do portal FFA levels remain increased 8 weeks after stopping short-term HFD? It was previously suggested that increased adipose tissue lipolysis is an important source of FFA promoting hepatic lipid accumulation.<sup>8</sup> In addition, TNF $\alpha$  stimulates lipolysis in adipocytes.<sup>16</sup> Hence, increased TNF $\alpha$  expression in mesenteric WAT may have promoted FFA release in HFD RF mice. Such effect may be limited to the mesenteric fat depot since short-term HFD did not affect lipolytic function of systemically drained epididymal WAT.<sup>6</sup> Consistently, systemic FFA concentrations were not different between HFD RF and chow-fed mice, suggesting similar degrees of lipolysis in systemically drained fat depots. It remains to be determined whether increased mesenteric lipolysis in HFD RF mice evolved during recovery feeding or whether it was already present at the end of the HFD challenge. The observed rise of TNF $\alpha$  expression in mesenteric adipose during short-term HFD<sup>5</sup> hints toward the latter possibility.

The finding of similar body weight after a short-bout of HFD is in line with some<sup>5,17,18</sup> but not all studies<sup>1–4</sup> examining the effect of short-term HFD on metabolism in mice. Possibly, differences in dietary composition, housing conditions, genetic background or age at dietary

intervention may have contributed to observed differences in body weight gain. Of note, body as well as fat depots weights was trend wise higher in HFD RF compared to solely chow-fed mice, further supporting the notion that a short-term HFD may persistently impact on metabolism in C57BL/6J mice. As food intake and energy expenditure were not assessed, it remains unclear whether increased energy input and/or decreased energy output were responsible for the slight increase in body weight of HFD RF mice.

In conclusion, a short-term HFD has long-lasting effects on glucose tolerance as well as on hepatic lipid accumulation in mice.

## Materials and Methods

### Animals

C57BL/6J mice were originally obtained from The Jackson Laboratory and then bred in our own facility. At the age of 12 weeks animals were fed *ad libitum* with standard rodent diet (chow) or HFD (D12331, Research Diets, New Brunswick, USA) for 7 d. Thereafter, all mice were fed with chow diet for 8 weeks. HFD consisted of 58% of calories derived from fat, 25.5% from carbohydrate and 15.5% from protein. All protocols conformed to the Swiss animal protection laws and were approved by the Cantonal Veterinary Office in Zurich, Switzerland.

### Intra-peritoneal glucose tolerance test (ipGTT)

Mice were injected intra-peritoneally with 2 g/kg body weight glucose after overnight fasting as previously described.<sup>19</sup> Blood glucose concentration was measured with a Glucometer (Accu-Check Aviva, Roche Diagnostics, Rotkreuz, Switzerland) with blood from tail-tip bleedings.

### Determination of plasma free fatty acids

Systemic and portal blood was sampled in mice that were fasted for 5 hours. Plasma free fatty acid (FFA) levels were determined as described elsewhere.<sup>20</sup>

### RNA extraction and quantitative reverse transcription-PCR (RT-PCR)

Total RNA was extracted using RNeasy Lipid Tissue Mini Kit (Qiagen, Basel, Switzerland) and concentration was determined spectrophotometrically (Nanodrop 1000; Nanodrop Technologies, Boston, MA). 1 µg of RNA was reverse transcribed with PrimeScriptReverse Transcriptase (Takara Bio Europe, Saint-Germain-en-

Laye, France). Taqman (Takara Bio Europe) was used for real-time PCR amplification. The following PCR primers (Applied Biosystems, Rotkreuz, Switzerland) were used: TNF- $\alpha$  Mm00443258\_m1, MCP-1 Mm00441242\_m1, Ela3b Mm00840378\_m1, FAS Mm00662319\_m1, Ppar $\alpha$  Mm00627559\_m1, Srebp1 Mm00550338\_m1, Scd1 Mm01197142\_m1, Cpt1 Mm00550438\_m1, Acox1 Mm00443579\_m1. Relative gene expression was obtained after normalization to 18sRNA (Applied Biosystems), using the formula  $2^{-\Delta\Delta C_p}$ .<sup>21</sup>

### Liver triglyceride and total lipid determination

Liver tissue (20–30 mg) was homogenized in PBS and lipids were extracted in a chloroform-methanol (2:1) mixture. Total liver lipids were determined by a sulfolipid phosphovanillin reaction as previously described.<sup>22</sup> Liver triglyceride levels were determined in 50 mg of liver tissue according to the method of Bligh and Dyer<sup>23</sup> and quantified with an enzymatic assay (Roche Diagnostics, Rotkreuz, Switzerland).

### Data analysis

Statistical analyses were performed using Student's *t* test. *p*-Values < 0.05 were considered significant. All error bars represent SEM.

### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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